Novel Derivatization Reagent, (2,4,5-Trimethyl-3-thienyl)acetic Acid, for Electrogenerated Tris(2,2′-bipyridine)ruthenium(III) Chemiluminescence Detection

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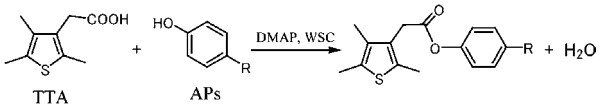
A novel derivatization reagent for $Ru(bpy)_3^{3+}$ (bpy = 2,2′-bipyridine) chemiluminescence (CL) detection has been developed. The derivatization reagent has a thiophene ring for CL reaction and a carboxylic group for derivatization reaction. The derivatized alkylphenols with this reagent were determined by a HPLC–CL detection system.

The $Ru(bpy)_3^{3+}$ CL system is useful as a selective and sensitive detection method in analytical chemistry.[1] Therefore, a wide range of analytical applications have been reported.[2] A variety of derivatization reagents such as divinyl sulfone,[3] methylmalonic acid,[4] diketene,[5] and acetaldehyde[6] have been used to extend this CL detection method to a wider range of compounds. Above all, a number of derivatization reagents possessing an aliphatic tertiary amine moiety were proposed because the $Ru(bpy)_3^{3+}$ CL detection system can sensitively detect aliphatic tertiary amines.[7–11] However, tertiary amine-type derivatization reagents exhibit following problems. First, it is necessary to control the reaction pH because the CL intensity of amine compounds is greatly affected by the reaction pH. Second, biological samples contain various compounds having an amine moiety. As a result, these compounds cause interfering peaks in the detection system using amine-type labeling reagents. Third, tertiary amines are known to strongly interact with residual silanol groups on silica-based reversed-phase columns to cause broadened and skewed peaks in chromatography.

The authors recently showed that the α-position-dialkylated thiophene derivatives, such as 2,5-dimethylthiophene, react with $Ru(bpy)_3^{3+}$ to emit strong CL.[12] In this paper, we report a novel derivatization reagent based on the CL reaction of $Ru(bpy)_3^{3+}$ with thiophene derivatives. As one application of the developed reagent, a HPLC determination method for measurement of alkylphenols (APs) has been proposed (Scheme 1).

Chemicals and solutions used were as follows. $Ru(bpy)_3Cl_2 \cdot 6H_2O$ was prepared in our laboratory. 3-(Cyanomethyl)-2,4,5-trimethylthiophene and 4-nonylphenol (mixture of compounds with branched side-chain) were obtained from Tokyo Kasei (Tokyo, Japan). 4-*n*-Butylphenol, 4-(1,1-dimethylhexyl)-phenol, 4-*n*-octylphenol, 4-*n*-nonylphenol, bisphenol A, and 4-dimethylaminopyridine (DMAP) were obtained from Wako pure chemical (Osaka, Japan). A standard stock solution of thirteen APs was obtained from Kanto Kagaku (Tokyo, Japan). 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (WSC) was obtained from Dojindo (Kumamoto, Japan). All other chemicals were of guaranteed grade and used without further purification. The labeling reagent, (2,4,5-trimethyl-3-thienyl)acetic acid (TTA), was synthesized from 3-(cyanomethyl)-2,4,5-trimethylthiophene at one-step reaction according to a procedure described in literature.[13,14] The TTA-derivative of 4-*n*-octylphenol (TTA-OP) was synthesized as one of the standard TTA-derivatized APs in order to examine the optimal conditions of derivatization and chemiluminescence reactions.



Scheme 1. Derivatization reaction scheme of APs with TTA.

A HPLC assembly consisted of a GL Science PU611 pump (GL Science, Tokyo, Japan), a Ryeodyne 7125 sample injector (20 or 500 μL), and a Chromolith Performance RP-18e column (100 × 4.6 mm i.d., Merck, Darmstadt, Garmany). For flow injection analysis (FIA) experiments, the column was removed from the system. An eluting (carrier) solution for examination of derivatization conditions was a 10 mM phosphate buffer–acetonitrile = 2:8 and delivered at a flow rate of 0.8 mL/min. A 0.5 mM $Ru(bpy)_3^{2+}$ solution was prepared by dissolving a weighed quantity of $Ru(bpy)_3Cl_2 \cdot 6H_2O$ in a 10 mM sulfuric acid. The $Ru(bpy)_3^{2+}$ solution was delivered at a flow rate of 0.3 mL/min and oxidized to $Ru(bpy)_3^{3+}$ by the controlled-current electrolysis method (50 μA, Galvanostat Comet 3000). The eluent and $Ru(bpy)_3^{3+}$ solution were mixed and pumped continuously through the spiral flow cell in a comet 3000 ECL detector (Comet, Kawasaki, Japan). Chromatograms were recorded with a Hitachi D-2500 Chromato-Integrator (Hitachi, Tokyo, Japan). The eluent and the $Ru(bpy)_3^{2+}$ solution were purged with a Shodex degass (Showa Denko, Tokyo, Japan). All connecting PTFE tubing was 0.5 mm i.d.

The derivatization reactions were performed according to a procedure described by Morita and Konishi.[7] To decide the optimal derivatization conditions, some preliminary experiments were performed by the HPLC system. The final derivatization procedure was as follows. A 20 μL of 300 mM DMAP solution in acetonitrile, a 10 μL of 50 mM TTA solution in acetonitrile, and a 10 μL of 50 mM WSC solution in acetonitrile were added to a 250 μL of APs sample solution prepared with acetonitrile. After mixing, the mixture was allowed for 2 h at room temperature. A 20 μL of methanol was firstly added to the mixture in order to stop the reaction and then a 300 μL of 50 mM phosphoric acid was added to the mixture in order to acidify. A 500-μL aliquot of the resulting mixture was injected into the HPLC system.

To develop the suitable CL detection conditions of TTA-derivatives, some preliminary experiments were performed with the FIA system. Although the CL intensity increased with increasing reaction pH, the signal-to-noise ratio degreased with

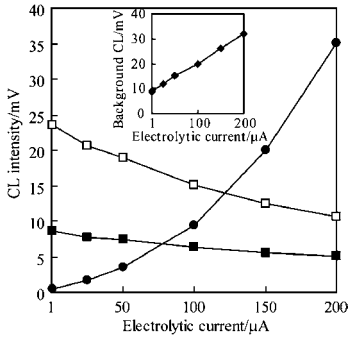


Figure 1. Effect of the electrolytic current on the CL intensity. Injection volume, 20 μL. Sample: TTA (■), 1.0 μM; TTA-OP (□), 1.0 μM; DMAP (●), 0.10 mM. Inset: effect of the electrolytic current on the background CL intensity.

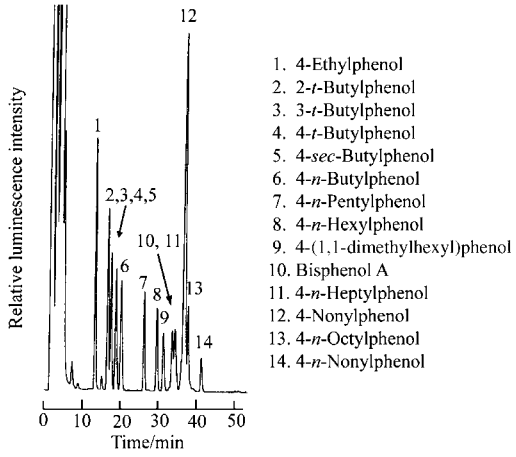


Figure 2. Chromatogram of the derivatives of APs. Sample: 50 ng/mL each except for 4-nonylphenol (0.50 μg/mL). Eluent; (A) 10 mM phosphoric acid–(B) acetonitrile (gradient program: 0–10 min, 50% A; 10–25 min, 60% A; 25–35 min, 70% A; 35–50 min, 80% A; 50–60 min, 100% A; 60–70 min, 50% A).

increasing reaction pH because hydroxide ions react with $Ru(bpy)_3^{3+}$ to cause background noise. As a result, the best signal-to-noise ratio was obtained at around pH 2. The effect of the electrolytic current of an electrochemical reactor on the CL intensity was shown in Figure 1. In general, the CL intensity increases with increasing the electrolytic current owing to an increase in the amount of $Ru(bpy)_3^{3+}$. In fact, the CL intensity of DMAP, which has a tertiary amine moiety, and the background CL increased. However, the CL intensities of TTA and TTA-OP increased with decreasing the electrolytic current down to at least 1 μA. The reason of the result is expected that the rate of this chemiluminescent reaction is very fast.[12] Therefore, the flow rate of carrier was made fast under the 1 μA of electrolytic current. The CL intensity was increased up to a flow late of 1.6 mL/min. These determination conditions, high acidity, low electrolytic current, and high flow rate, lead to more selective detection because such conditions are disadvantageous for the measurement of other detectable compounds such as aliphatic amines.

Table 1. Detection limits and relative standard deviation

Compound	D.L./ng mL^{-1}[a]	R.S.D./%[b]
4-*n*-Buthylphenol	1.4	1.3
4-(1,1-dimethylhexyl)phenol	3.5	2.5
Bisphenol A	2.3	1.3
4-Nonylphenol	5.7	4.3
4-*n*-Octylphenol	1.5	2.9
4-*n*-Nonylphenol	4.4	5.1

[a]Signal-to-noise ratio of 3. [b]Six operation at 50 ng/mL.

Figure 2 shows a typical chromatogram of TTA-derivatives of fourteen APs obtained with a stepwise elution method. Table 1 shows the detection limits (D.L.) and the relative standard deviations (R.S.D.) of six APs obtained with the proposed method. The linear ranges of these compounds were at least 2 orders of magnitude.

In conclusion, a novel derivatization reagent for $Ru(bpy)_3^{3+}$ CL detection was developed. As the optimal detection conditions for TTA-derivatives were exclusive, use of this reagent should enable more selective detection.

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References and Notes

1 *Electrogenerated Chemiluminescence*, ed. by A. J. Bard, Marcel Dekker, New York, **2004**.
2 B. A. Gorman, P. S. Francis, N. W. Barnett, *Analyst* **2006**, *131*, 616.
3 K. Uchikura, M. Kirisawa, A. Sugii, *Anal. Sci.* **1993**, *9*, 121.
4 K. Uchikura, *Anal. Sci.* **2000**, *16*, 453.
5 K. Uchikura, *Chem. Lett.* **2003**, *32*, 98.
6 J. Li, Q. Yan, Y. Gao, H. Ju, *Anal. Chem.* **2006**, *78*, 2694.
7 H. Morita, M. Konishi, *J. Liq. Chromatogr. Relat. Technol.* **2002**, *25*, 2413.
8 H. Morita, M. Konishi, *Anal. Chem.* **2002**, *74*, 1584.
9 H. Morita, M. Konishi, *Anal. Chem.* **2003**, *75*, 940.
10 X.-B. Yin, B. Qi, X. Sun, X. Yang, E. Wang, *Anal. Chem.* **2005**, *77*, 3525.
11 X.-B. Yin, Y. Du, X. Yang, E. Wang, *J. Chromatogr. A* **2005**, *1091*, 158.
12 H. Kodamatani, Y. Komatsu, S. Yamazaki, K. Saito, *Anal. Sci.* **2007**, *23*, 407.
13 A solution of 3-(cyanomethyl)-2,4,5-trimethylthiophene (1 g) in 50-mL ethanol was added to a solution of KOH (1.4 g) in 20-mL water. The mixture was refluxed for 30 h. The reaction mixture was evaporated to exclude ethanol, and then the mixture was acidified with hydrochloric acid. The resulting solution was extracted with chloroform three times. The organic layer was evaporated in vacuo. The residue was dissolved with a small portion of acetonitrile and purified by column chromatography. TTA: mp 106–107 °C. Anal. calcd; C, 58.67; H, 6.56%. Found C, 58.58; H, 6.54%. ^{1}H NMR ($CDCl_3$): δ 2.03 (3H, s), δ 2.28 (3H, s), δ 2.33 (3H, s), δ 3.49 (2H, s).
14 J. R. Ruhoff, *Org. Synth.* **1943**, Coll. Vol. *II*, 292.

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